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## ORIGINAL ARTICLE

# Genetic variability of the *PRNP* gene in goat breeds from Northern and Southern Italy

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## Keywords

disease resistance, genotyping, goat, *PRNP*, scrapie.

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## Abstract

**Aims:** To determine the variability of the prion protein gene (*PRNP*) in goats from Northern and Southern Italy.

**Methods and results:** Genomic DNA isolated from goat blood was polymerase chain reaction (PCR)-amplified for the coding region of the *PRNP* gene and then sequenced. In total, 13 polymorphic sites were identified: G37V, T110P, G127S, M137I, I142M, I142T, H143R, R154H, P168Q, T194P, R211Q, Q222K and S240P (substitutions I142T and T194P are novel) giving rise to 14 haplotypes. Clear frequency differences between Northern and Southern breeds were found and confirmed by genetic distance analysis.

**Conclusions:** Differences in allele distribution were found between Northern and Southern goats, in particular regarding the M142 and K222 alleles, possibly associated to scrapie resistance; phyllogeographical analysis supported the idea that Northern and Southern breeds may be considered as separate clusters.

**Significance and impact of the study:** In Italy only limited studies have been carried out on caprine *PRNP* genotype distribution; this study is important to fill this lack of information. Moreover the finding of significant differences among allele distributions in Northern and Southern goats, especially if involved in modulating resistance/susceptibility, need to be carefully considered for the feasibility of selection plans for resistance to scrapie.

## Introduction

Scrapie is a fatal, infectious, neurodegenerative disease that occurs in sheep and goats. Like bovine spongiform encephalopathy (BSE) in cattle and Creutzfeld-Jakob Disease (CJD) in humans, scrapie is a transmissible spongiform encephalopathy (TSE). TSEs are characterized by the accumulation in the central nervous system of an abnormal isoform (PrP<sup>Sc</sup>) of a host-encoded cellular prion protein (PrP<sup>C</sup>) (Prusiner 1991).

In sheep, scrapie appears to be entirely an infectious disease in which genetic susceptibility plays an important role. This susceptibility is largely determined by genotypes

of the prion protein gene (*PRNP*). *PRNP* haplotypes valine/arginine/glutamine (VRQ) and alanine/arginine/glutamine (ARQ) at codons 136, 154, 171 respectively, are associated with high susceptibility to scrapie, whereas the ARR allele has been linked to decreased susceptibility or even resistance (Belt *et al.* 1995; Bossers *et al.* 1996; Hunter *et al.* 1996, 1997). Accordingly, the European Union (EU) has implemented programmes for the genetic control of scrapie susceptibility in sheep populations. In compliance with EU Decision 2003/100/EC, each member state has introduced a breeding programme to increase the frequency of the resistance-associated ARR allele in sheep populations. A similar breeding

programme has not been devised for controlling TSE in goats, because no clear association between PRNP genotypes and resistance to scrapie has been demonstrated in this species yet.

The described amino acid substitutions in caprine PRNP are: V21A, L23P, G37V, G49S, W102G, T110N, T110P, G127S, L133Q, M137I, I142M, H143R, N146S, N146D, R151H, R154H, P168Q, R211Q, I218L, Q220H, Q222K and S240P (Goldmann *et al.* 1996, 1998, 2004; Wopfner *et al.* 1999; Billinis *et al.* 2002; Agrimi *et al.* 2003; Zhang *et al.* 2004; Kurosaki *et al.* 2005; Acutis *et al.* 2006; Papasavva-Stylianou *et al.* 2007). The W102G substitution has been solely found in combination with a PrP variant containing only three instead of the usual five octapeptide repeats (Goldmann *et al.* 1998). The presence of methionine at codon 142 and the three repeats/G102 variant were associated with increased incubation periods after experimental challenge with BSE and scrapie strains (Goldmann *et al.* 1996, 1998). Protection against natural scrapie infection is thought to be offered by the R143 and H154 variants in Greek goats (Billinis *et al.* 2002) and by the presence of serine or aspartic acid at codon 146 in the goats of Cyprus (Papasavva-Stylianou *et al.* 2007). In a case-control study on 177 goats from six scrapie outbreaks in Italy, we recently found a possible association with resistance to scrapie of the glutamine (Q) to lysine (K) mutation at codon 222 (Acutis *et al.* 2006). Similar results were reported by Vaccari *et al.* (2006) in another Italian scrapie-affected goat herd.

As investigation into the relationships between caprine PRNP haplotypes and resistance to scrapie continues, knowledge of the PRNP genotype distribution in goat breeds has become increasingly important. Estimating the frequency of candidate alleles in a population is a preliminary step in understanding the feasibility of a selection programme. Before implementing their selection programmes, each EU member state completed a survey on the genetic variation of the PRNP locus in sheep breeds (Decision 2002/1003/EC).

Studies on PRNP allele frequencies in goats have been carried out in Greece (Billinis *et al.* 2002), United Kingdom (Goldmann *et al.* 1996, 2004), Cyprus (Papasavva-Stylianou *et al.* 2007), Japan (Kurosaki *et al.* 2005) and China (Zhang *et al.* 2004). The aim of this study was to describe genetic variability at the PRNP locus in some goat breeds reared in Northern and Southern Italy.

## Materials and methods

### Specimen collection

Blood samples were collected from 300 goats, belonging to 81 herds, of four goat breeds commonly reared in

Northern Italy (Saanen  $n = 69$ ; Camosciata delle Alpi  $n = 84$ ; Roccaverano  $n = 70$ ; Valdostana  $n = 77$ ) and from 178 goats, belonging to 19 herds, of six breeds (Garganica  $n = 58$ ; Maltese  $n = 25$ ; Cilento  $n = 10$ ; Ionica  $n = 27$ ; Red Mediterranean  $n = 28$ ; Girgentana  $n = 10$ ) (<http://www.agraria.org/ovini.htm>; <http://www.tiho-hannover.de/einricht/zucht/eaap/>) and a cross-bred group ( $n = 20$ ) from unknown mating schemes reared in Southern Italy.

### Genetic analysis

Genomic DNA was isolated using the Thermo LabSystems KingFisher instrument (Promega, Madison, WI, USA) and the ChargeSwitch Sheep Blood Kit (Invitrogen, Carlsbad, CA, USA). Polymerase chain reaction (PCR) amplification of the open reading frame of the caprine PRNP gene was performed according to the protocol described previously (Acutis *et al.* 2004), using the primers p8(+) (5'-ATGGTGAAAAGCCACATAGG-3') and p9(-) (5'-TATCCTACTATGAGAAAAATGAGG-3') (Bossers *et al.* 1996). The polymorphisms were detected by direct DNA sequencing on both strands of the PCR products using the Big Dye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and a four-capillary ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Sequencing primers were p8(+), p61(+) (5'-AACCAACATGAAGCATGTGG-3'), p60(-) (5'-GATAGTAACGGTCCTCATAG-3') and p9(-) (Belt *et al.* 1995). The primers hybridize on the target PRNP DNA at codons 1–7, 109–116, 147–154 and 249–257, respectively (GenBank accession number AJ000739). Sequence alignment was carried out using the SeqScape software v2.5 (Applied Biosystems).

### Cloning

The PCR products were purified by means of the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and cloned into the pDrive Cloning Vector using a Qiagen PCR Cloning kit. The cloned fragments were cycle-sequenced using an ABI PRISM 310 Genetic Analyzer by the Big Dye Terminator v1.1 Cycle sequencing kit (Applied Biosystems). Sequencing on both strands was performed using the two M13 vector primers. The obtained sequences were compared and aligned by the ClustalW program (Thompson *et al.* 1994).

### Data analysis

The chi-square test for independence or Fisher's exact test was performed in order to look for differences in allele frequencies. This was done by considering each allele once

and comparing its frequencies among the Northern breeds and between Northern and Southern breeds. Statistical analysis of the Southern breeds was performed only with Garganica, Maltese, Ionica and Red Mediterranean because there were too few Girgentana and Cilento individuals in the sample; nevertheless these two breeds were considered in the comparison between Northern and Southern goats. A *P*-value <0.05 was considered statistically significant.

The *F*-statistics were computed across all breeds using the AMOVA (analysis of molecular variance) design implemented by the Arlequin 3.11 software (Excoffier *et al.* 2005). In addition, molecular variance was partitioned at hierarchical level according to two geographical groups, North and South Italy; hierarchical AMOVA was performed both among breeds within geographical groups and between groups. Significant deviations from the null hypothesis were tested with 1000 permutations.

The genetic relationships between the breeds at the PRNP locus were estimated using the chord distance (*D<sub>c</sub>*) assuming that all gene frequency changes were because of genetic drift alone and that population sizes did not remain constant in all breeds over time (Cavalli-Sforza and Edwards 1967). The matrix of the genetic distances between pairs of breeds was used to construct a neighbour-joining (NJ) tree as implemented by Populations software (Langella 1999). Significant deviations from the null values were tested with 1000 permutations using Génétix software (Belkhir 2004).

## Results

In total, 13 polymorphic sites were identified giving rise to 14 alleles (Table 1). Alleles derived from known muta-

tions were inferred on the basis of previous reports (Goldmann *et al.* 1996, 2004; Billinis *et al.* 2002; Kurosaki *et al.* 2005; Acutis *et al.* 2006; Vaccari *et al.* 2006; Papasavva-Stylianou *et al.* 2007). The amino acid substitution I142T (t>c in second codon position) and T194P (a>c in first codon position) are novel (submitted to Genbank under accession numbers EF192309 and EF192310); the corresponding alleles (8 and 12) were obtained by cloning and sequencing. The three-octarepeat variant was not found in any of the examined animals.

## Northern breeds

Only nine alleles were found in this group; the allele frequencies are reported in Table 2. Alleles were found combined in 28 different genotypes (data not shown). Silent mutations were found at codon 42 (ccg>cca) (200 goats, 62 of which were homozygotes) and at codon 138 (agc > agt) (228 goats, 95 of which were homozygotes).

Allele 2 was the most frequent in all breeds except in Valdostana, followed by allele 1 (corresponding to the wild-type sheep). The most frequent allele in Valdostana was allele 1, followed in the descending order by alleles 7 and 2. Predominant genotypes (frequency in parenthesis) were: 2/2 (0.17) and 1/2 (0.13) in Camosciata delle Alpi; 2/2 (0.4) and 1/2 and 2/13 (both 0.13) in Saanen; 2/2 (0.24) and 2/13 (0.17) in Roccaverano; 1/2 and 1/7 were the most common genotypes in Valdostana (0.22 and 0.21, respectively).

Alleles 5 (G127S) and 7 (I142M) reached the highest frequency in the Valdostana breed; differences among the frequencies of these alleles were statistically significant. Allele 10 (R154H) was only present in Camosciata delle Alpi and Roccaverano; the chi-square test demonstrated that this difference was significant.

**Table 1** Alleles in the goat population sample

Allele	Amino acid position												
	37	110	127	137	142	143	154	168	194	211	222	240	
1	G	T	G	M	I	H	R	P	T	R	Q	S	
2	—	—	—	—	—	—	—	—	—	—	—	P	
3	V	—	—	—	—	—	—	—	—	—	—	—	
4	—	P	—	—	—	—	—	—	—	—	—	—	
5	—	—	S	—	—	—	—	—	—	—	—	P	
6	—	—	—	I	—	—	—	—	—	—	—	P	
7	—	—	—	—	M	—	—	—	—	—	—	P	
8	—	—	—	—	T	—	—	—	—	—	—	—	
9	—	—	—	—	—	R	—	—	—	—	—	P	
10	—	—	—	—	—	—	H	—	—	—	—	—	
11	—	—	—	—	—	—	—	Q	—	—	—	P	
12	—	—	—	—	—	—	—	—	P	—	—	—	
13	—	—	—	—	—	—	—	—	—	Q	—	—	
14	—	—	—	—	—	—	—	—	—	—	K	—	

**Table 2** Allele frequencies shared across Northern breeds and *P*-values

Allele	Camosciata				<i>P</i> -value
	delle Alpi	Saanen	Roccaverano	Valdostana	
1	0.298	0.188	0.257	0.340	*
2	0.327	0.587	0.443	0.205	***
4	0.000	0.007	0.000	0.000	n.s.
5	0.012	0.014	0.021	0.064	*
7	0.089	0.072	0.057	0.282	***
10	0.113	0.000	0.036	0.000	***
11	0.000	0.000	0.007	0.000	n.s.
13	0.137	0.102	0.136	0.096	n.s.
14	0.024	0.030	0.043	0.013	n.s.
Total	1.000	1.000	1.000	1.000	

n.s., not significant.

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

Alleles 4 (T110P) and 11 (P168Q) were found at a very low rate and only in one breed (Saanen and Roccaverano, respectively). Allele 13 (R211Q) reached a relatively high frequency across all the Northern breeds, without showing a significant variation among them. Frequency of allele 14 (Q222K) was low in all the breeds.

### Southern breeds

The allele frequencies in the Southern breeds are reported in Table 3. Twelve alleles and 27 different genotypes were found (data not shown). Silent mutations were found at codon 42 (118 goats, 30 of which were homozygotes) and at codon 138 (148 goats, 60 of which were homozygotes). Two novel alleles (8 and 12) were found; they were present in two Cilento goats and in one cross-bred goat, respectively.

Allele 2 was the most frequent, followed by alleles 14 (Q222K), 1 and 10. The other alleles showed low frequencies or were absent in some breeds. Predominant genotypes (frequencies in parentheses) were: 2/2, 1/2 and 2/14 (0.27, 0.19 and 0.13, respectively). No significant differences in allele frequencies were found, except for allele 3 (V37), which was well represented in Maltese.

### Northern vs Southern breeds

The allele frequencies of Northern and Southern goats are listed in Table 4, along with the corresponding *P*-values. Alleles 2 (P240) and 1 reached the highest frequencies in both groups and were significantly more frequent in Southern and Northern goats, respectively.

Alleles 5 (S127) and 13 (Q211) were found only in Northern goats; alleles 3 (V37), 6 (I137), 8 (T142), 9

**Table 3** Allele frequencies shared across Southern breeds and *P*-values

Allele	Red				Southern cross-bred	<i>P</i> -value
	Garganica	Maltese	Ionica	Mediterranean		
1	0.103	0.100	0.130	0.214	0.125	n.s.
2	0.510	0.520	0.592	0.607	0.525	n.s.
3	0.034	0.140	0.056	0.000	0.000	*
4	0.026	0.000	0.000	0.000	0.000	n.s.
6	0.000	0.000	0.019	0.000	0.000	n.s.
7	0.026	0.000	0.000	0.000	0.000	n.s.
9	0.017	0.040	0.037	0.054	0.050	n.s.
10	0.112	0.060	0.074	0.053	0.050	n.s.
11	0.000	0.020	0.019	0.018	0.000	n.s.
12	0.000	0.000	0.000	0.000	0.025	n.s.
14	0.172	0.120	0.073	0.054	0.225	n.s.
Total	1.000	1.000	1.000	1.000	1.000	

n.s., not significant.

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

**Table 4** Allele frequencies in Northern and Southern goats and *P*-values

Allele	Northern breeds	Southern breeds	<i>P</i> -value
1	0.274	0.126	***
2	0.382	0.531	***
3	0.000	0.040	***
4	0.002	0.008	n.s.
5	0.028	0.000	***
6	0.000	0.003	n.s.
7	0.128	0.008	***
8	0.000	0.006	n.s.
9	0.000	0.034	***
10	0.040	0.076	**
11	0.002	0.008	n.s.
12	0.000	0.003	n.s.
13	0.118	0.000	***
14	0.028	0.157	***
Total	1.000	1.000	

n.s., not significant.

\**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

(R143) and 12 (P194) were detected only in Southern goats. However, the differences were statistically significant only for alleles 3, 5, 9 and 13. Allele 7 (M142) was statistically more frequent in the Northern goats; alleles 10 (H154) and 14 (K222) were significantly more frequent in the Southern goats. The other alleles reached low frequencies in both Southern and Northern goat populations; no statistically significant differences between the two groups were observed.

### F-statistics and genetic distances

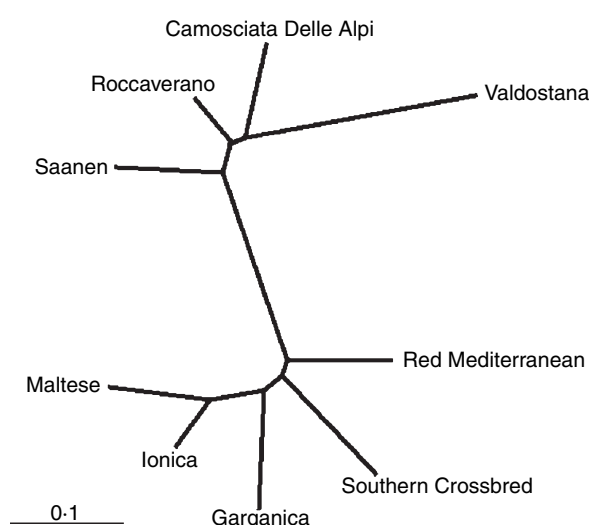
Variation among breeds, as estimated by the global  $F_{ST}$  index, was highly significant ( $F_{ST} = 0.063$ ,  $P < 0.001$ ) (Table 5). This finding indicates that a substantial proportion of global diversity is because of genetic distances among breeds. In addition, Northern and Southern groups showed significant differences ( $F_{CT} = 0.049$ ,  $P < 0.01$ ). Some genetic diversity was present within groups ( $F_{SC} = 0.038$ ,  $P < 0.001$ ), but only the Northern breeds were globally different ( $F_{ST} = 0.054$ ,  $P < 0.001$ ) while the Southern breeds appeared to be more homogeneous ( $F_{ST} = 0.004$ ,  $P > 0.05$ ).

The *Dc*-based tree provided a discerning picture, as breeds belonging to the same geographical location clustered tightly (Fig. 1). Among the Northern breeds, the Roccaverano shared a similar allele distribution with both Camosciata delle Alpi and the Saanen, whereas the Valdostana appeared to be quite a different population. In the Southern breeds, only the Red Mediterranean showed significant differences with respect to the Garganica and the Maltese (Table 6).

Structure	Source of variation	Variation (%)	Fixation indices
No structure (nine breeds)	Among breeds	6.32	$F_{ST} = 0.063^{***}$
	Within breeds	93.68	
No structure (Northern breeds only)	Among breeds	5.39	$F_{ST} = 0.054^{***}$
	Within breeds	94.61	
No structure (Southern breeds only)	Among breeds	0.38	$F_{ST} = 0.004$ n.s.
	Within breeds	99.62	
Geographical, two groups (Northern and Southern breeds)	Among breeds	3.59	$F_{SC} = 0.038^{***}$
	within groups		
	Between groups	4.95	$F_{CT} = 0.049^{**}$
	Within breeds	91.46	

n.s., not significant.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Figure 1** Neighbour-joining tree based on the  $D_c$  genetic distance.

## Discussion

This study provides data about polymorphisms of the caprine *PRNP* locus in Italian goats. Some alleles have been reported as possibly associated with susceptibility or resistance to scrapie and they were found to be differently distributed in the study populations. Two main *PRNP* alleles (1 and 2) are present in Italian goats, characterized by the presence of Ser or Pro at codon 240. The S240 allele is homologous to the wild-type allele of the ovine *PRNP* gene but is less frequent than the P240 allele in Italian goats, except for the Valdostana breed. This distribution is also reported in Greek and Cyprus goats (Billinis *et al.* 2002; Papasavva-Stylianou *et al.* 2007), while S240 is the most frequent allele in UK goats. No clear association between mutations at codon 240 and scrapie susceptibility has been demonstrated so far (Goldmann *et al.* 1996; Billinis *et al.* 2002; Acutis *et al.* 2006; Papasavva-Stylianou *et al.* 2007).

**Table 5** Partitioning of genetic variation with and without implementation of geographic grouping of breeds

**Table 6** Matrix of  $D_c$  distances and their significances

2	3	4	5	6	7	8	9	
0.008	0.029	0.035	0.034	0.077	0.063	0.093	0.081	1
	0.012	0.034	0.058	0.062	0.048	0.077	0.066	2
		0.036	0.062	0.069	0.054	0.084	0.070	3
			0.119	0.137	0.117	0.150	0.137	4
				0.021	0.030	0.021	0.023	5
					0.015	0.008	0.027	6
						0.030	0.019	7
							0.034	8
n.s.	***	***	***	***	***	***	***	1
	n.s.	***	***	***	***	***	***	2
		***	***	***	***	***	***	3
			***	***	***	***	***	4
				n.s.	**	n.s.	n.s.	5
					n.s.	n.s.	n.s.	6
						*	n.s.	7
							n.s.	8

1, Camosciata delle Alpi; 2, Roccaverano; 3, Saanen; 4, Valdostana; 5, Garganica; 6, Ionica; 7, Red Mediterranean; 8, Maltese; 9, Southern cross-bred group; n.s., not significant.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Most other alleles found in this study are present at low frequency in both Northern and Southern breeds or seem to be exclusive to one of the two groups: alleles 3 (V37), 4 (P110), 5 (S127), 6 (I137), 8 (T142) and 12 (P194). None of the first four alleles have been associated with scrapie (Acutis *et al.* 2006; Vaccari *et al.* 2006). The substitution M>T at codon 137 has been described in Dutch Swifter and Icelandic ovine population (Bossers *et al.* 1996; Thorgeirsdottir *et al.* 1999). T137 may interfere with the efficient conversion from PrP<sup>C</sup> to PrP<sup>Sc</sup> (Bossers *et al.* 2000). T142 and P194 are described here for the first time; hence the risk related to them is unknown. T142 has been described in sheep (Zhou *et al.* 2005).

Allele 9 (R143) is absent in Northern breeds and its frequency in Southern goats is low, similar to frequencies

reported for the UK and Japanese goats (Goldmann *et al.* 1996; Kurosaki *et al.* 2005). In some Greek and Chinese goat breeds, however, this allele is frequent (Billinis *et al.* 2002; Zhang *et al.* 2004). It is thought to be moderately protective against scrapie in Greece (Billinis *et al.* 2002). Discordant results have been obtained in Italy, where no association with resistance was found by Acutis *et al.* (2006) and a weak protective role of the genotype H/R 143 has been suggested by Vaccari *et al.* (2006).

Allele 11 (Q168) is also quite rare in this study sample: the 168 codon is polymorphic in sheep (P168L) and is associated with a prolonged incubation period after challenge with BSE (Goldmann *et al.* 2006). Whether a similar protective role of Q168 does exist for scrapie in goats is not clear, but the report of a scrapie case in a Q168 animal (Acutis *et al.* 2006) indicates that only partial resistance is likely to be associated with this polymorphism.

Allele 13 (Q211) reaches a relatively high frequency in all Northern breeds. A single case of natural scrapie in a goat heterozygous for this allele was reported in Italy (Acutis, personal communication); the relative risk associated with this mutation could not be estimated.

Allele 10 (H154) is present in nearly all breeds but occurs significantly more frequently in Southern goats. It is also present in sheep: in Irish and Icelandic sheep it seems to be resistant with incomplete dominance (Thorgeirsdottir *et al.* 1999; O'Doherty *et al.* 2002); in Italy and Spain it is related to susceptibility perhaps owing to the presence of geographically different scrapie strains (Mutinelli *et al.* 2003; Acin *et al.* 2004; Acutis *et al.* 2004). In goats, H154 is held to be moderately protective against scrapie in Greek goats (Billinis *et al.* 2002) while analogous Italian studies related this mutation to full susceptibility (Acutis *et al.* 2006) or to a delay in the progression of the disease (Vaccari *et al.* 2006). This variant is of particular interest because it has been shown to be associated with high susceptibility to the atypical scrapie strain Nor98 in sheep (Buschmann *et al.* 2004; Moum *et al.* 2005; Sounders *et al.* 2006). It has also been detected in Nor98-affected goats in France (Le Dur *et al.* 2005), Switzerland (Seuberlich *et al.* 2007) and Italy (unpublished data) suggesting that it could confer high susceptibility to Nor98 in goats as well. If so, the data from our study show that the Southern populations could be more at risk of contracting this disease.

Two alleles found in this study have been associated with partial resistance to scrapie in goats: alleles 7 (M142) and 14 (K222). Interestingly, they have a significantly different distribution in Northern and Southern breeds, the former being present only in Northern breeds and the latter significantly more frequent in Southern breeds. Valdostana is distinct among the Northern breeds in that

it has a significantly high frequency of allele 7, which is the second most frequent allele in this group. It is also highly frequent in UK goats (Goldmann *et al.* 1996), but has never been detected in Mediterranean area goat breeds such as Greek and Cyprus goats (Billinis *et al.* 2002; Papasavva-Stylianou *et al.* 2007). This allele has been related to a prolonged incubation period in goats challenged with BSE and scrapie strains showing a protective role against TSE (Goldmann *et al.* 1996).

Allele 14 has been associated with resistance to scrapie in Italy (Acutis *et al.* 2006; Vaccari *et al.* 2006). The protective role of lysine at caprine codon 222 is also supported by the fact that at human PRNP codon 219, which is homologous to caprine codon 222, the E219K mutation is present; K219 is, in fact, the only known protective factor against sporadic CJD (Shibuya *et al.* 1998). This allelic variant could be a valuable candidate in the framework of suitable breeding programmes for scrapie resistance in goats. Selection of this allele would be also feasible because it is present across all breeds, even if its frequency in Northern breeds is significantly lower.

The genetic distance analysis revealed that the Northern group seems to be less homogeneous than the Southern group. The Valdostana was found to differ markedly from other Northern breeds. Similar results were obtained in a study that assessed goat population structure by single nucleotide polymorphisms and screened 35 genes in eight different Italian goat breeds (Pariset *et al.* 2006). In this investigation, the Valdostana showed a high proportion of individuals assigned to a peculiar group. In contrast, the Roccaverano shares a similar allele distribution with both the Camosciata delle Alpi and the Saanen, with which it has been heavily crossed in recent years (Porter 2002). These two most selected breeds showed different genetic characteristics.

A comparison between the Northern and Southern groups shows a different distribution of the PRNP alleles. This finding may be interpreted as a consequence of the phylogeographical structure of the Italian breeds. A genetic structure related to geographic distribution has been demonstrated in European and Middle Eastern goat breeds using microsatellite and SNP markers, the PRNP gene included (Cañón *et al.* 2006; Pariset *et al.* 2006). These investigations showed that Italian goats may be separated into two clusters: Northern breeds belonging to the Central-Northern Europe group and Southern breeds belonging to the Central-Mediterranean group. As pointed by Cañón *et al.* (2006), the phylogeographical structure in goat populations is more obvious than in other domestic species. In fact, gene flow among breeds has been restricted by spatial isolation, whereas the use of herd books is rarely practiced, so that geographical clines are maintained that predate breed formation.

Possible future breeding programmes should take these differences into account and would be more or less feasible, according to the genetic structure of the populations of interest.

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